

What is claimed is:

1. A method for generating a directionally linked recombinant nucleic acid molecule, the method comprising contacting:

a) a topoisomerase-charged first double stranded (ds) nucleic acid molecule, comprising a first topoisomerase covalently bound at or near a first end, and a second topoisomerase covalently bound at or near a second end, said first end further comprising a first 5' overhang, and said second end further comprising a blunt end, a 3' thymidine overhang, or a second 5' overhang; and

b) a second ds nucleic acid molecule, comprising a first blunt end and a second end, wherein the first blunt end comprises at its 5' terminus, a nucleotide sequence complementary to the first 5' overhang,

under conditions such that the nucleotide sequence complementary to the first 5' overhang can selectively hybridize to the first 5' overhang,

whereby the first topoisomerase can covalently link the 3' terminus of the first end of the first ds nucleic acid molecule with the 5' terminus of the first end of the second ds nucleic acid molecule, and

whereby the second topoisomerase can covalently link the 3' terminus of the second end of the first nucleic acid molecule to the 5' terminus of the second end of the second ds nucleic acid molecule, thereby generating a directionally linked nucleic acid molecule.

2. The method of claim 1, wherein the second end of the first ds nucleic acid molecule comprises a blunt end, and the second end, of the second ds nucleic acid molecule comprises a blunt end.

3. The method of claim 1, wherein the second end of the topoisomerase-charged first ds nucleic acid molecule comprises a 3' thymidine overhang, and the second end of the second ds nucleic acid molecule comprises a 3' adenosine overhang.

4. The method of claim 1, wherein the topoisomerase-charged, first ds nucleic acid molecule comprises a second 5' overhang at the second end, and the second ds nucleic acid comprising at the second end, a nucleotide sequence complementary to the second 5' overhang.

5. The method of claim 1, wherein the first ds nucleic acid molecule is a vector.

6. The method of 5, wherein the topoisomerase-charged first ds nucleic acid molecule is a cloning vector.

7. The method of 6, wherein the topoisomerase-charged first ds nucleic acid molecule is an expression vector.

8. The method of claim 1, further comprising introducing the directionally-linked recombinant nucleic acid molecule into a cell.

9. The method of claim 8, wherein the cell is a eukaryotic cell.

10. The method of claim 9, wherein the cell is a mammalian cell.

11. A cell produced by the methods of claim 8.

12. A transgenic non-human organism generated from the cell of claim 11.

13. The method of claim 8, wherein the cell is a bacterium.

14. The method of claim 1, wherein the second ds nucleic acid molecule comprises an amplification product.



21. A method for generating a directionally linked recombinant nucleic acid molecule, the method comprising contacting:

- a) a first precursor double stranded (ds) nucleic acid molecule comprising a first end, which comprises at the 5' terminus, a first 5' target sequence, and at the 3' terminus, a topoisomerase recognition site; and a second end which comprises at the 3' terminus, a topoisomerase recognition site;
  - b) a second ds nucleic acid molecule comprising a first blunt end and a second end, wherein the first blunt end comprises at the 5' terminus a nucleotide sequence complementary to the 5' target sequence; and
  - c) a topoisomerase specific for the topoisomerase recognition site,
- under conditions that allow topoisomerase activity, and that allow hybridization of the first 5' target sequence and the nucleotide sequence complementary to the target sequence, thereby generating a directionally linked recombinant nucleic acid molecule.

22. The method of claim 21, wherein the second end of the first precursor ds nucleic acid becomes a blunt end upon cleavage by the topoisomerase, and the second end of the second ds nucleic acid molecule is a blunt end.

23. The method of claim 21, wherein the second end of the first precursor ds nucleic acid molecule comprises a 3' thymidine extension upon cleavage by the topoisomerase, and the second end of the second ds nucleic acid molecule comprises a 3' adenosine overhang.

24. The method of claim 21, wherein the first precursor ds nucleic acid molecule comprises a second 5' target sequence located at the second end and the second ds nucleic acid molecule comprises at the second end, a nucleic acid sequence complementary to the second 5' target sequence.

25. The method of claim 24, wherein the first precursor ds nucleic acid molecule is a vector.

26. The method of claim 25, wherein the vector is an expression vector.

27. The method of claim 21, further comprising introducing the directionally-linked recombinant nucleic acid molecule into a cell.

28. The method of claim 21, wherein the first precursor ds nucleic acid molecule comprises an expression control element and the second ds nucleic acid molecule comprises an open reading frame, wherein in the directionally linked recombinant nucleic acid molecule, the expression control element is operatively linked to the open reading frame.

29. The method of claim 21, wherein the second ds nucleic acid molecule comprises an amplification product.

30. The method of claim 21, wherein the topoisomerase is a type IB topoisomerase.

31. The method of claim 21, wherein the second ds nucleic acid molecule comprises one of a plurality of second ds nucleic acid molecules.

32. The method of claim 31, wherein said plurality of second ds nucleotide molecules comprises a cDNA library.

33. A recombinant nucleic acid molecule produced by the method of claim 21.

34. A method for generating a directionally linked recombinant nucleic acid molecule, the method comprising contacting:

a) a topoisomerase-charged first double stranded (ds) nucleic acid molecule, comprising a first topoisomerase covalently bound to the 3' terminus of a first end of the ds nucleic acid molecule, said first end further comprising a first 5' overhang; and

b) a second ds nucleic acid molecule, comprising a first blunt end and a second end, wherein the first blunt end comprises a 5' nucleotide sequence complementary to the first 5' overhang,  
under conditions such that the 5' nucleotide sequence of the first blunt end can selectively hybridize to the first 5' overhang,

whereby the first topoisomerase can covalently link the 3' terminus of the first end of the first ds nucleic acid molecule with the 5' terminus of the first end of the second ds nucleic acid molecule.

35. The method of claim 34, further comprising contacting the topoisomerase-charged first ds nucleic acid molecule and the second ds nucleic acid molecule with a third ds nucleic acid molecule,

wherein a first end of the third nucleic ds acid molecule comprises a 5' overhang and a second topoisomerase covalently bound at the 3' terminus, and

wherein the second ds nucleic acid molecule further comprises a second blunt end, which comprises a 5' nucleotide sequence complementary to the second 5' overhang, and

wherein the contacting is performed under conditions such that the 5' nucleotide sequence of the second blunt end of the second ds nucleic acid can selectively hybridize to the 5' overhang of the first end of the third ds nucleic acid molecule,

whereby the second topoisomerase can covalently link the 3' terminus of the first end of the third ds nucleic acid molecule with the 5' terminus of the second blunt end of the second ds nucleic acid molecule.

36. The method of claim 35, wherein the first ds nucleic acid molecule is directionally linked to the second ds nucleic acid molecule and, thereafter, the third ds nucleic acid molecule is directionally linked to the second ds nucleic acid molecule.

37. The method of claim 34, wherein the first ds nucleic acid molecule is operatively linked to the second ds nucleic acid molecule.

38. The method of claim 34, wherein the first ds nucleic acid comprises an expression control element and the second ds nucleic acid comprises an open reading frame.

39. The method of claim 35, wherein the first ds nucleic acid molecule comprises an expression control element, the second ds nucleic acid molecule comprises an open reading frame, and the third ds nucleic acid molecule encodes a peptide,

wherein, in the directionally linked recombinant nucleic acid molecule, the expression control element is operatively linked to the open reading frame, and the second ds nucleic acid molecule is operatively linked to the third ds nucleic acid molecule, and

wherein the second ds nucleic acid molecule is operatively linked to the third ds nucleic acid molecule encode a fusion protein comprising the open reading frame and the peptide.

40. The method of claim 39, wherein the peptide comprises a tag.

41. An isolated double stranded (ds) nucleic acid molecule, comprising a first topoisomerase covalently bound at a 3' terminus of a first end, and a second topoisomerase covalently bound at a 3' terminus of a second end, said first end further comprising a first 5' overhang and said second end further comprising a blunt end, a 3' thymidine overhang, or a second 5' overhang, wherein said first 5' overhang is different from said second 5' overhang.

42. The ds nucleic acid molecule of claim 41, wherein the second end comprises a blunt end.

43. The ds nucleic acid molecule of claim 41, wherein the second end comprises a single 3' thymidine overhang.

44. The ds nucleic acid molecule of claim 41, wherein the second end comprises a second 5' overhang.

45. The ds nucleic acid molecule of claim 41, wherein the first 5' overhang comprises the nucleotide sequence 5'-GGTG-3'.

46. The ds nucleic acid molecule of claim 41, wherein the ds nucleic acid molecule is a vector.

47. The ds nucleic acid molecule of claim 46, wherein the vector further comprises a recombinase site.

48. The ds nucleic acid molecule of claim 47, wherein the vector further comprises a lox site.

49. The ds nucleic acid molecule of claim 46, wherein the ds nucleic acid molecule is a cloning vector.

50. The ds nucleic acid molecule of claim 49, wherein the ds nucleic acid molecule is an expression vector.



51. The ds nucleic acid molecule of claim 48, wherein the first end and the second end are adjacent sequences of a nucleotide sequence encoding a selectable marker.

52. The ds nucleic acid molecule of claim 46, wherein the vector is a pUni/V5-His version A vector (SEQ ID NO:16).

53. The ds nucleic acid molecule of claim 52, wherein the 5' overhang of the first end comprises 5'-GGTG-3', and wherein the second end is a blunt end, which comprises a topoisomerase recognition site at the 3' terminus.

54. The ds nucleic acid molecule of claim 46, wherein the vector is a pCR<sup>®</sup>2.1 vector.

55. The ds nucleic acid molecule of claim 54, wherein the 5' overhang of the first end comprises 5'-GAAT-3', and wherein the second end is a blunt end, which comprises a topoisomerase recognition site at the 3' terminus.

56. A composition, comprising:

a) a first ds nucleic acid molecule comprising a first end and a second end, wherein the first end comprises a 5' overhang and a topoisomerase covalently bound at the 3' terminus, and

b) a second ds nucleic acid molecule comprising a first blunt end and a second end, wherein the first blunt end comprises a first 5' nucleotide sequence, which is complementary to the first 5'-overhang, and a first 3' nucleotide sequence complementary to the first 5' nucleotide sequence.

57. The composition of claim 56, wherein the first 5' nucleotide sequence of the first blunt end of the second ds nucleic acid molecule is hybridized to the first 5' overhang of the first end of the first nucleic acid molecule, and the first 3' nucleotide sequence of the first blunt end of the second ds nucleic acid molecule is displaced.

58. The composition of claim 56, wherein the first ds nucleic acid molecule further comprises a second 5' overhang at the second end,  
wherein the second end of the second ds nucleic acid molecule further comprises a second 5' nucleotide sequence, which is complementary to the second 5' overhang, and a second 3' nucleotide sequence complementary to the second 5' nucleotide sequence.

59. A kit containing the ds nucleic acid molecule of claim 1.

60. The kit of claim 59, wherein the nucleic acid molecule is a vector.

61. The kit of claim 59, further comprising an expression control element.

62. A kit, comprising

a) a first double stranded (ds) nucleic acid molecule, which comprises a first topoisomerase covalently bound at a 3' terminus of a first end, and a second topoisomerase covalently bound at a 3' terminus of a second end,

said first end further comprising a first 5' overhang and said second end further comprising a blunt end, a 3' thymidine overhang, or a second 5' overhang, wherein said first 5' overhang is different from said second 5' overhang; and

b) a plurality of second ds nucleic acid molecules, wherein each ds nucleic acid molecule in the plurality comprises a first blunt end, and wherein the first blunt end comprises a 5' nucleotide sequence complementary to the first 5' overhang of the first ds nucleic acid molecule.

63. The kit of claim 62, wherein the second ds nucleic acid molecules in the plurality comprise transcriptional regulatory elements, translational regulatory elements, or a combination thereof.

64. The kit of claim 62, wherein the second ds nucleic acid molecules in the plurality comprise nucleotide sequences encoding a peptide.

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